

We claim:

1. An isolated nucleic acid comprising a sequence encoding a wth3 protein.
2. The nucleic acid of Claim 1 which encodes the amino acid sequence comprising SEQ ID NO:12 from about amino acid residue number 1 to about amino acid residue number 254.
3. The nucleic acid of Claim 1 which comprises the nucleotides of SEQ ID NO:11.
4. An isolated nucleic acid encoding an immunogenic fragment of wth3 having from 7 to 25 contiguous amino acid residues of SEQ ID NO:12 and comprising at least 3 amino acid residues of SEQ ID NO:19 or SEQ ID NO:20 or SEQ ID NO:21 or SEQ ID NO:22 or SEQ ID NO:23 or SEQ ID NO:24.
5. An isolated nucleic acid comprising a sequence encoding the amino acid sequence of SEQ ID NO:27 from about amino acid residue number 1 to about amino acid residue number 208.
6. The nucleic acid of Claim 5 which comprises SEQ ID NO:26.
7. A recombinant DNA comprising the nucleic acid of any one of Claims 1 to 6 operably linked to regulatory control nucleic acid sequences which can effect expression of said nucleic acid sequence in a host cell.
8. An expression vector comprising the recombinant DNA of Claim 7.
9. A host cell comprising the expression vector of Claim 8.
10. The host cell of Claim 9 wherein said host cell is a eukaryotic or prokaryotic cell.
11. A method of producing a recombinant wth3 protein or immunogenic fragment thereof, which process comprises:
 - a) culturing the host cell of Claim 9 in a culture medium under conditions suitable for expression of the wth3 protein or immunogenic fragment thereof in said host cell, and
 - b) recovering said recombinant protein or immunogenic fragment thereof from said host cell or said culture medium.
12. A wth3 protein or immunogenic fragment thereof prepared by the method of Claim 11.
13. A substantially purified protein comprising wth3.
14. A substantially purified immunogenic fragment of wth3 having 7 to 25 contiguous amino acid residues of SEQ ID NO:12 and comprising at least 3 amino acid residues of SEQ ID

NO:19 or SEQ ID NO:20 or SEQ ID NO:21 or SEQ ID NO:22 or SEQ ID NO:23 or SEQ ID NO:24.

15. A substantially purified protein or polypeptide comprising the immunogenic fragment of Claim 14.

16. A substantially purified immunogenic fragment of wth3 capable of generating antibodies which bind to wth3 and not to rab6.

17. A substantially purified protein comprising SEQ ID NO:27 from about amino acid residue number 1 to about amino acid residue 208.

18. An isolated nucleic acid comprising a sequence of at least about 15 nucleotides of SEQ ID NO: 10 which is capable of hybridizing to a nucleic acid segment of a WTH3 gene under stringent hybridization conditions wherein said nucleic acid fails to hybridize to a nucleic acid segment of a RAB6 gene.

19. The isolated nucleic acid of Claim 18 which comprises at least 20 adjacent nucleotides of SEQ ID NO:10.

20. The isolated nucleic acid of Claim 18 which comprises SEQ ID NO:7 from about nucleotide number 1 to about nucleotide number 573.

21. An antibody which binds selectively to wth3 protein wherein the affinity of said antibody for wth3 protein is greater than the affinity of said antibody for rab6 protein.

22. A method of producing a hybridoma which secretes an antibody that binds to wth3 protein wherein the affinity of said antibody for wth3 protein is greater than the affinity of said antibody for rab6 protein, which comprises:

- a) immunizing an animal with wth3 protein or a polypeptide fragment thereof;
- b) obtaining lymphoid cells from the immunized animal;
- c) fusing the lymphoid cells and an immortalizing cell to produce hybrid cells; and
- d) selecting hybrid cells which produce antibody that:
 - i) specifically binds to wth3 protein; and
 - ii) binds to rab6 protein with an affinity that is lower than the affinity of said antibody for wth3 protein.

23. A method for identifying wth3 protein in a test sample, which comprises contacting the test sample with an anti-wth3 antibody or fragment thereof that specifically binds to wth3 protein with an affinity that is greater than the affinity of said antibody for rab6 protein and forms a complex therewith.

24. The method of claim 23 which further comprises contacting a control sample with said anti-wth3 antibody or fragment thereof.

25. A method for identifying wth3 protein in a test sample, which comprises

- a) contacting the test sample with a first anti-wth3 antibody under conditions such that wth3 protein binds to said first antibody and forms a complex therewith;
- b) contacting the test sample with a second anti-wth3 antibody that specifically binds to wth3 protein such that a second complex is formed therewith;
- c) contacting the complex formed in step (b) with an indicator reagent comprising a signal generating compound and capable of binding to said first or second anti-wth3 antibody; and
- d) detecting the signal generating compound, thus detecting the wth3 protein

wherein at least one of said first and said second anti-wth3 antibodies binds to wth3 protein with an affinity that is greater than the affinity of said antibody for rab6 protein.

26. A kit for the specific assay of wth3 protein in a test sample comprising an antibody or antibody fragment capable of binding specifically to wth3 protein with an affinity that is greater than the affinity of said antibody or antibody fragment for rab6 protein.

27. A method for detecting whether a DNA sequence is hypomethylated or hypermethylated in the region of genomic DNA which comprises WTH3 that is present in a test sample of cells which comprises:

- a) cleaving genomic DNA isolated from said test sample of cells with a master restriction enzyme to generate a cleaved test-cell DNA;
- b) hybridizing a probe to said cleaved test-cell DNA to form a hybridization complex, wherein said probe comprises a nucleic acid which hybridizes to a region of said test-cell DNA which is adjacent to said DNA sequence; and
- c) determining the size of the hybridization complex;

wherein said master restriction enzyme cleaves a nonmethylated DNA sequence but does not cleave a methylated DNA sequence.

28. The method of claim 27 wherein said region of genomic DNA which comprises WTH3 is within 10 kb of the transcribed WTH3 sequence.

29. The method of claim 27 which further comprises:

- a) cleaving genomic DNA isolated from a control sample of cells with said master restriction enzyme to generate a cleaved control-cell DNA;

- b) hybridizing said probe to said cleaved control-cell DNA to form a control-hybridization complex; and
- c) determining the size of the control-hybridization complex.

30. A kit for identifying whether a DNA sequence in a test sample is hypomethylated or hypermethylated in the region of genomic DNA which comprises WTH3, said kit comprising a nucleic acid hybridization assay probe capable of forming a detectable hybrid with WTH3 DNA and not with RAB6 DNA under stringent hybridization conditions.

31. A method for detecting WTH3 mRNA in a test sample which comprises:

- a) contacting test sample RNA with a nucleic acid probe which is capable of forming a hybrid with WTH3 mRNA, and
- b) detecting nucleic acid probe-WTH3 mRNA hybrids;

wherein said mRNA and said probe are contacted under stringent hybridization conditions selected to prevent hybridization of said probe to RAB6 mRNA.

32. The method of claim 31 which further comprises detecting WTH3 mRNA in a control sample.

33. The method of claim 31 which further comprises:

- a) contacting said test sample RNA with a second nucleic acid probe which is capable hybridizing to RNA which is constitutively expressed; and
- b) comparing said WTH3 mRNA-nucleic acid probe hybrids with constitutively expressed-second nucleic acid probe hybrids.

34. A kit for detecting WTH3 mRNA in a test sample, which comprises a nucleic acid hybridization probe capable of forming a detectable hybrid with WTH3 mRNA under stringent hybridization conditions selected to prevent hybridization of said probe to RAB6 mRNA.

35. The kit of claim 34 which further comprises a control RNA having a known amount of WTH3 mRNA.

36. The kit of claim 34 which further comprises a nucleic acid probe capable of forming a detectable hybrid with constitutively expressed RNA.

37. A method for detecting WTH3 mRNA in a test sample, which comprises making cDNA from said WTH3 mRNA and detecting said cDNA, wherein RAB6 mRNA is not detected.

38. The method of claim 37 which further comprises detecting WTH3 mRNA in a control sample.

39. The method of claim 37 which further comprises detecting constitutively expressed RNA in said test sample and comparing said WTH3 mRNA with said constitutively expressed RNA.

40. The method of claim 37 wherein detection of said cDNA comprises an amplification reaction.

41. A kit for detecting WTH3 mRNA comprising an oligonucleotide capable of hybridizing to WTH3 mRNA in a test sample and priming reverse transcription of WTH3 cDNA and comprising primers capable of amplifying said WTH3 cDNA, wherein RAB6 mRNA is not detected.

42. A method for detecting whether a DNA sequence is hypomethylated or hypermethylated in the region of genomic DNA which comprises RAB6 that is present in a test sample of cells which comprises:

- a) cleaving genomic DNA isolated from said test sample of cells with a master restriction enzyme to generate a cleaved test-cell DNA;
- b) hybridizing a probe to said cleaved test-cell DNA to form a hybridization complex, wherein said probe comprises a nucleic acid which hybridizes to a region of said test-cell DNA which is adjacent to said DNA sequence; and
- c) determining the size of the hybridization complex;

wherein said master restriction enzyme cleaves a nonmethylated DNA sequence but does not cleave a methylated DNA sequence.

43. The method of claim 42 wherein said region of genomic DNA which comprises RAB6 is within 10 kb of the transcribed RAB6 sequence.

44. The method of claim 42 which further comprises:

- a) cleaving genomic DNA isolated from a control sample of cells with said master restriction enzyme to generate a cleaved control-cell DNA;
- b) hybridizing said probe to said cleaved control-cell DNA to form a control-hybridization complex; and
- c) determining the size of the control-hybridization complex.

45. A kit for identifying whether a DNA sequence in a test sample is hypomethylated or hypermethylated in the region of genomic DNA which comprises RAB6, said kit comprising a nucleic acid hybridization assay probe capable of forming a detectable hybrid with RAB6 DNA and not WTH3 DNA under stringent hybridization conditions.

46. A method for measuring RAB6 mRNA in a test sample which comprises:
- contacting said test sample RNA and control RNA with a nucleic acid probe which is capable of forming a hybrid with RAB6 mRNA; and
 - comparing test sample RNA-nucleic acid probe hybrids and control RNA-nucleic acid hybrids.
47. A method for measuring RAB6 mRNA in a test sample which comprises:
- contacting said test sample RNA with a first nucleic acid probe which is capable of forming a hybrid with RAB6 mRNA;
 - contacting said test sample RNA with a second nucleic acid probe which is capable of forming a hybrid with RNA which is constitutively expressed; and
 - comparing test sample RNA-first nucleic acid probe hybrids with constitutively expressed RNA-second nucleic acid probe hybrids.
48. A kit for measuring RAB6 mRNA in a test sample, which comprises a first nucleic acid hybridization probe capable of forming a detectable hybrid with RAB6 mRNA under stringent hybridization conditions, and
- a second nucleic acid hybridization probe capable of forming a detectable hybrid with constitutively expressed RNA, or
 - a control RNA.
49. A method for measuring RAB6 mRNA in a test sample, which comprises making cDNA from said RAB6 mRNA, and;
- making cDNA from constitutively expressed RNA in said test sample, or
 - making cDNA from RAB6 mRNA in a control sample;
- and comparing said cDNAs.
50. The method of claim 49 wherein comparing said cDNAs comprises an amplification reaction.
51. A kit for measuring RAB6 mRNA in a test sample, said kit comprising a first oligonucleotide capable of hybridizing to RAB6 mRNA and priming reverse transcription of cDNA, and;
- a second oligonucleotide capable of hybridizing to a constitutively expressed RNA and priming reverse transcription of cDNA, or
 - a control RNA.

52. A method for detecting mRNA having homology to WTH3 in a test sample which comprises:

- a) contacting test sample RNA with a nucleic acid probe which is capable of forming a hybrid with WTH3 mRNA; and
- b) detecting nucleic acid probe-WTH3 homolog mRNA hybrids

wherein said mRNA and said probe are contacted under stringent hybridization conditions selected to prevent hybridization of said probe to RAB6 mRNA.

53. The method of claim 52 which further comprises detecting mRNA having homology to WTH3 in a control sample.

54. The method of claim 52 which further comprises:

- a) contacting said test sample RNA with a second nucleic acid probe which is capable of hybridizing to RNA which is constitutively expressed;
- b) comparing said WTH3 homolog mRNA-nucleic acid probe hybrids with constitutively expressed RNA-second nucleic acid probe hybrids.

55. A kit capable of detecting mRNA homologous to WTH3 in a test sample, which comprises a nucleic acid hybridization probe capable of forming a detectable hybrid with WTH3 mRNA under stringent hybridization conditions selected to prevent hybridization of said probe to RAB6 mRNA.

56. The kit of claim 55 which further comprises a control RNA having a known amount of WTH3 mRNA.

57. The kit of claim 55 which further comprises a nucleic acid probe capable of forming a detectable hybrid with constitutively expressed RNA.

58. A method for detecting mRNA homologous to WTH3 in a test sample, which comprises making cDNA from said mRNA and detecting said cDNA, wherein RAB6 mRNA is not detected.

59. The method of claim 58 which further comprises detecting mRNA homologous to WTH3 in a control sample.

60. The method of claim 58 which further comprises detecting constitutively expressed RNA in said test sample and comparing said mRNA homologous to WTH3 with said constitutively expressed RNA.

61. The method of claim 58 wherein detection of said cDNA comprises an amplification reaction.

62. A kit for detecting mRNA homologous to WTH3 comprising an oligonucleotide capable of hybridizing to WTH3 mRNA in a test sample and priming reverse transcription of WTH3 cDNA and comprising primers capable of amplifying WTH3 cDNA, wherein RAB6 mRNA is not detected.

63. A method for measuring mRNA having homology to RAB6 in a test sample which comprises:

- a) contacting test sample RNA and control RNA with a nucleic acid probe which is capable of forming a hybrid with RAB6 mRNA; and
- b) comparing test sample RNA-nucleic acid probe hybrids and control RNA-nucleic acid hybrids.

64. A method for measuring mRNA homologous to RAB6 in a test sample which comprises:

- a) contacting said test sample RNA with a first nucleic acid probe which is capable of hybridizing to RAB6 mRNA;
- b) contacting said test sample RNA with a second nucleic acid probe which is capable of hybridizing to RNA which is constitutively expressed; and
- c) comparing test sample RNA-first nucleic acid hybrids with constitutively expressed RNA-second nucleic acid hybrids.

65. A kit for measuring mRNA homologous to RAB6 in a test sample, which comprises a first nucleic acid hybridization probe capable of forming a detectable hybrid with RAB6 mRNA under stringent conditions, and

- i) a second nucleic acid hybridization probe capable of forming a detectable hybrid with constitutively expressed RNA, or
- ii) a control RNA.

66. A method for measuring mRNA homologous to RAB6 in a test sample which comprises making cDNA from said mRNA, and;

- a) making cDNA from constitutively expressed RNA in said test sample, or
 - b) making cDNA from mRNA homologous to RAB6 in a control sample;
- and comparing said cDNAs.

67. The method of claim 66 wherein comparing said cDNAs comprises an amplification reaction.

68. A kit for measuring mRNA homologous to RAB6 in a test sample, which comprises a first oligonucleotide capable of hybridizing to RAB6 mRNA and priming reverse transcription of cDNA, and;

- a) a second oligonucleotide capable of hybridizing to a constitutively expressed RNA and priming reverse transcription of cDNA, or
- b) a control RNA.

69. A method for increasing drug sensitivity in a multiply drug resistant cell which comprises insertion of a nucleic acid comprising WTH3, RAB6C or RAB6 and expression thereof.

70. A recombinant expression vector suitable for increasing drug sensitivity in a multiply drug resistant host cell comprising a nucleotide as claimed in Claim 1 or Claim 5 and a regulatory sequence operatively linked to the nucleic acid.

71. A method of determining the suitability of a therapeutic for treatment of a cancer, wherein the therapeutic is substantially ineffective for treatment of cells which have acquired multidrug resistance, which comprises:

providing a test sample from the cancer;

measuring expression of WTH3 mRNA, or RAB6C mRNA, or RAB6 mRNA, or a homologous mRNA in the test sample;

comparing expression of the mRNA in the test sample with expression of the mRNA in a control sample; and

determining whether expression of the mRNA in the test sample is reduced relative to expression of the mRNA in the control sample.

72. The method of Claim 71 wherein the therapeutic substance is doxorubicin.

73. The method of Claim 71 wherein the therapeutic substance is vincristine.

74. The method of Claim 71 wherein the cancer is a leukemia.

75. The method of Claim 71 wherein the control sample is from doxorubicin sensitive cells.

76. The method of Claim 71 wherein the control sample is from MCF7 cells.

77. A method for determining whether a substance increases sensitivity of a cell to a therapeutic which comprises:

providing a test cell which overproduces wth3 or rab6c or rab6 or a homolog thereof;

incubating the substance with the test cell and the therapeutic; and
comparing the response of the test cell in the presence of the therapeutic and the
substance to the response of the test cell in the presence of the therapeutic and the absence
of the substance.

78. The method of Claim 77 wherein the therapeutic is an anti-cancer drug.

79. The method of Claim 77 wherein the therapeutic is substantially ineffective for
treatment of cells which have acquired multidrug resistance.

80. The method of Claim 77 wherein the therapeutic is doxorubicin.